

THE CHEMISTRY OF THE STEM OF DERRIS ULIGINOSA, *BENTH.*

AN EASTERN FISH POISON.

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Introductory.

The number of plants, or parts of plants, which are used in various countries for the purpose of stupefying or killing fish, and are therefore designated as "fish poisons," is very considerable. Greshoff, in his work entitled: *Mededeelingen uit's Lands Plantentuin*, x, 1893, has described more than two hundred such plants, and quite a number of others have since been brought to notice. Although the plants of this character belong to a variety of natural orders, they appear to be most largely represented by the *Leguminosæ*, *Euphorbiaceæ*, and *Sapindaceæ*. The nature of the substances to which the poisonous action has been attributed is likewise extremely variable, although as yet they have been, for the most part, very incompletely investigated. Many of the plants in question contain substances belonging to the group of saponins, whilst some are assumed to owe their activity to other glucosides, to alkaloids and so-called bitter principles (such as picrotoxin), or to resins, volatile oils, the production of hydrocyanic acid, etc. Compare, for example, Watts' *Dictionary of the Economic Products of India*, Vol. vi., Part I, p. 312; Greshoff, *Ber. d. deutsch. Chem. Ges.*, 1890, 23, p. 3538, and *Pharm. Journ.*, 1890, p. 559; Pool, *Chem. Centralblatt*, 1898, i., p. 520; Sillevoldt, *Archiv der Pharm.*, 1899, p. 595; Schaer, *Chem. Zeitung*, 1901, p. 922; Rosenthaler, *Archiv der Pharm.*, 1902, p. 57.)

One of the most recent observations relating to the use of a plant as a fish poison is embodied in a paper by H. M. Kyle, communicated to the Royal Society, December 12, 1901, and reported in *Nature*, May 8, 1902, entitled: "On the action of the Spurge (*Euphorbia hiberna*, L.) on Salmonoid Fishes." These observations are of considerable interest, especially in connection with the views of the author respecting the constituent of the plant to which the poisonous action on fishes is attributed, and on account of their bearing upon the subject of this investigation the brief report referred to may be quoted:

"It has been known for some years that the Irish peasantry employed a simple method of procuring salmon and trout through the agency of the Spurge (*E. hiberna*, L.). The plant cut into small pieces and pounded with stones, or simply trampled upon at some convenient spot on a river, forms an emulsion in the water which, being swept downward into the pools, carries death to all fishes in its course. The fatality thus produced seems to have been enormous—80 to 100 salmon are reported to have been killed at one time, and again in the Bandon rivers 500 to 1,000 fish of various descriptions are said to have been poisoned during one season. In the light of the experiments to be

recorded presently, these statements do not seem exaggerated, for the Spurge-extract, even in small quantities, is almost as fatal to fishes as corrosive sublimate.*

"The fatal effect of the Spurge on fishes has been known in other countries besides Ireland, but to what ingredient or ingredients of the plant these effects are due seems never to have been investigated. The experiments described in the present paper throw considerable light upon the action of the Spurge, and open out to view some interesting problems."

"Chemical analysis of the Spurge-extract shows that it contains tannic acid. Experiments on the circulation in the lung and mesentery of the frog reveal a close similarity between the action of the Spurge-extract and of tannic acid. In the case of trout the similarity extends to the non-recovery of the fish in fresh water, after they have come under the influence of either Spurge-extract or tannic acid. The power of the Spurge-extract to produce fatal effects persists for several days without diminution. Twenty per cent. of the fresh extract is fatal within five minutes, whilst 0.01 per cent. takes 4 to 6 hours, and seems to be the smallest percentage which has fatal results.* In the case of fishes, death is considered to ensue from the inflammation of the gills and consequent stasis of the circulation, set up by the action of the tannic-acid component of the Spurge-extract. The fresh extract is calculated roughly to contain about 1 per cent. of tannic acid, but on this calculation the Spurge-extract is fatal within a shorter period than the corresponding quantity of tannic acid. Hence the percentage of tannic acid has been under-estimated, or some other substance or substances in the extract also aid in producing fatal effects."

In this connection the following reference to a similar use of another species of *Euphorbia* is of interest.

"*Euphorbia ocellata* of the Pacific coast is used as an antidote for snake bites, and is said to contain 2.82 per cent. of resin, besides gallo-tannic acid; whilst *E. cremocarpus* of the same region is employed for the purpose of catching fish in still ponds and streamlets and is said to contain a volatile oil, besides acid and resin." (*U. S. Dispensatory*, 18th Edit., p. 1651.)

The observation of Kyle is somewhat striking, inasmuch as it is believed to be the first instance in which the poisonous action of a plant on fishes has been attributed to the tannic acid it contains. Although this opinion respecting the cause of the toxicity appears to have been to some extent confirmed by experiments with tannic acid itself, it is highly probable, as he has indicated, that the toxic action is at least aided by the presence of other substances, for it is well known that many species of the genus *Euphorbia* are particularly characterized by the acrid resins they contain.

With regard to the class of plants to which the drug forming the subject of this investigation belongs, the following summary of available information may be recorded:

In Watts' *Dictionary of the Economic Products of India*, Vol. iii., p. 80 *Derris* is described as "a genus of arborescent climbers or trees, embracing

* Note.—The latter statement is ambiguous. A 20 per cent. solution of the extract was probably intended, but the quantity of water to which this was added is also not indicated.—P.

ing some 40 species, abundant in India, but according to the *Flora of British India* found 'belting the world' in the tropics."

Very little of an economic nature has been recorded regarding the Indian species, of which the following three are mentioned in the above work :

"*Derris elliptica*, Benth.—According to the Kew Report of 1877, the roots of this plant, steeped in water, afford a useful insecticide for gardening purposes. It is also used to kill fish. The Malays use the bark as one of the ingredients in their *Ipo* arrow-poison. *Derris robusta*, Benth.—The wood of this species may be used for tea boxes. *Derris scandens*, Benth.—The bark affords a coarse rope fibre."

The only species of *Derris* that has hitherto been subjected to any extended chemical examination is the above-mentioned *Derris elliptica*, Benth. This appears to have been first examined in 1890 by Greshoff, whose results were published in the work entitled: "Eerste verslag van het onderzoek naar de plantenstoffen van Ned Indie." A short account of this investigation is given in the *Ber. d. deutsch. Chem. Ges.*, 1890, 23, p. 3538, as also in the *Journ. Chem. Soc. Abst.*, Vol. 60, p. 335, *Pharm. Journ.*, December, 1890, p. 559, and *Proc. Amer. Pharm. Assoc.*, 1891, p. 655, from which the following is abstracted :

"The active principle of *Derris elliptica* is an acid resinous body to which the name derrid' has been given. Crude derrid melts at 61° C., but decomposes when heated to 60° C., giving off an odor resembling that of coumarin. Heated with caustic alkali, it yields salicylic acid and a little protocatechuic acid. It is associated in the root with a brown coloring matter—*derris-red*—derived from the tannin. The crude derrid, of which the root yields about five per cent., consists of a crystalline and an amorphous portion. These were separated by treating the substance with dilute spirit, subsequently with a very dilute alkali, and finally dissolving in chloroform. After distilling off the chloroform, the amorphous residue was heated with absolute alcohol, which afforded, on cooling, yellow circular crystals, melting at 190° C. with partial decomposition. The amorphous derrid remains in the mother liquor after the separation of the crystals. The exact relation of the crystalline to the amorphous derrid has not yet been determined. Derrid appears to be one of the most powerful fish poisons known. A solution containing only one five-millionth part stupefied gold fishes in a few minutes and killed them within half an hour."

A more recent examination of the same species of *Derris* has been made by H. E. Th. van Sillevoldt (*Archiv der Pharm.*, 1899, p. 595). The latter investigator describes the method of obtaining the so called derrid," which is essentially that given by Greshoff, and, although the substance was obtained only in an amorphous form, it was assigned the formula $C_{33}H_{30}O_{10}$. The crude "derrid" is stated to be accompanied by a substance crystallizing in light yellow needles, which melts at about 14° C., is insoluble in water, very sparingly soluble in cold alcohol, benzol, ether and petroleum ether, more readily in chloroform and acetic ether, and is not poisonous to fish. This crystalline substance is stated

to have the formula $C_{33}H_{28}O_9 \cdot \frac{1}{2} H_2O$, and was regarded as an "anhydroderrid." As a crystalline substance of the same composition was obtained by heating derrid in alcoholic solution with hydrochloric acid, it was assumed that it is thus artificially produced from the derrid, although the yield was but about 25 per cent. of the latter. Having supposed such a relationship to exist between derrid and anhydroderrid, and that the derrid must therefore contain at least two hydroxyl groups, an attempt was made to replace these by acetyl and benzoyl groups respectively, but with negative results. By the application of Zeisel's method to both derrid and anhydroderrid, results were obtained which led Sillevoldt to the further assumption that both of these substances contain three methoxyl groups. These deductions will be further considered in the experimental part of this paper.

The only chemical examination that has hitherto been made of *Derris uliginosa* appears to be that recorded in the "Pharmacographia Indica," Vol. i., p. 471, where the following statement occurs:

"A proximate analysis of the bark reveals the presence of a neutral crystalline principle, a wax and two resins in the ether extract; two coloring matters, an alkaloid and glucose in the alcoholic extract; an acrid glucoside allied to saponin, together with gum in the aqueous extract, and eight per cent. of mineral matter."

But little further information is given in the above work regarding these substances, and no reference is given to any other publication comprising experimental details or the methods employed for their separation and identification.

The material for the present investigation was obtained from Mr. F. L. Langdale (Retired Lieutenant, Royal Navy), of Wakaya, Fiji, who had kindly sent a quantity of the drug to Messrs. Burroughs, Wellcome & Co., of London. The botanical characters of the plant, and particularly the anatomy of the stem, have been very thoroughly studied in connection with this investigation by Mr. P. E. F. Perrédès, B. Sc., F. L. S., whose paper on the subject, with illustrations, is contained in the *Proc. Amer. Pharm. Assoc.*, Vol. 50, 1902, pp.

In a letter from Mr. Langdale, dated April, 1899, he noted the following observation respecting the action upon fish of this vine, known to the natives of Fiji as "Duva," when crushed and mixed with the enclosed waters of the reef lagoons: "The water after a time turns to a reddish hue and all the fish are killed. The natives use the vine largely for fishing, and although it stupefies or kills the fish it has no bad effects on their flesh."

Experimental.

The following preliminary tests were first made with the drug:

Ten grammes of the ground stem were extracted with Prollius' fluid, but this afforded no indication of the presence of an alkaloid.

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Forty grammes of the ground stem were extracted successively with various solvents, when the following percentages of extract, dried at 100° C., were obtained :

Petroleum (b. p. 40-60° C.)	0.373 gm. = 0.93 per cent.
Ether	0.131 gm. = 0.33 per cent.
Chloroform	0.111 gm. = 0.28 per cent.
Alcohol	1.118 gm. = 2.79 per cent.
Total	4.33 per cent.

All of these extracts were dark in color, of a resinous character, and, when treated with acidulated water, none of them gave any reaction for an alkaloid. The statement in the *Pharmacographia Indica*, previously referred to, that this drug contains an alkaloid, could thus not be confirmed, nor was any evidence of such a substance obtained in the subsequent examination of a large quantity of material.

Test for Saponin.—As it is likewise stated in the *Pharmacographia Indica* that the drug contains “an acrid glucoside allied to saponin,” a special search was made for a substance of this class, as follows :

Five hundred grammes of the ground stems were extracted with three successive portions of hot water. A dark reddish-brown liquid was obtained, which frothed considerably on agitation, but the froth was not very persistent. The liquid gave a yellowish color with ferric chloride and was precipitated by lead acetate, but much more abundantly by the basic salt. It slightly reduced Fehling’s solution, and to a much greater extent after boiling with an acid. The combined aqueous liquids were evaporated on a water bath to a small bulk, mixed with 50 grammes of magnesia, the mixture completely dried, and extracted in a Soxhlet with strong alcohol. From the alcoholic liquid there separated a considerable amount of colorless, needle-shaped crystals, which were entirely inorganic, and consisted chiefly of potassium nitrate with a little chloride. The alcohol was then distilled off, and the residue taken up with water, which precipitated a little resinous matter. The filtered liquid gave a precipitate with baryta water, which was due, however, to sulphate, and afforded no evidence of the presence of saponin. The frothing of the original aqueous liquid did not appear more strongly marked than in many solutions of vegetable extracts containing *gum*, and a considerable amount of the latter was observed to be present. There is, however, in one of the resins, as will be noted later on, a substance of glucosidal character which produces strong and persistent frothing when shaken with water, and which may be allied to saponin.

Test for Proteid.—As it was thought that the reputed poisonous action of the drug might be due to a proteid, 500 grammes of the ground stems were mixed with 2 liters of water, and allowed to macerate for two days.

The filtered liquid, either in its original state or when acidulated, gave no reaction with the usual proteid reagents, and it was therefore evident that no soluble substance of this class was present.

Determination of the Tannin.—With consideration of the interesting observation of Kyle, referred to in the first part of this paper, it seemed desirable to determine the amount of tannin present in the drug. For this purpose the bark alone was used, which in the piece of stem selected was found to correspond to 71 per cent. of the total weight of the latter. The method employed consisted in the repeated percolation of the aqueous extract through hide powder.

Twenty-five grammes of the ground bark were extracted with several successive portions of hot water, and the filtered liquid made up to the measure of 500 Cc. 50 Cc. of this liquid when evaporated, and the resulting extract dried in a steam oven until of constant weight, left a residue weighing 0.5390 gramme. The residue left by 50 Cc. of the liquid after treatment with hide powder, and likewise dried to a constant weight, amounted to 0.3058 gramme. The amount of substance which had combined with the hide powder was therefore 0.2332 gramme, and this would correspond to 9.3 per cent. of tannin in the bark. As the bark constituted 71 per cent. of the total weight of the stem, the amount of tannic matter in the particular specimen of the latter, assuming the woody portion to be free from tannin, as is apparently the case, would be 6.6 per cent. The tannin is the variety giving a greenish-black color with ferric chloride.

For the more complete examination of the constituents of the drug a larger quantity of material was treated in the following manner:

Three kilos of the ground stems were extracted with hot alcohol, and the alcohol subsequently distilled off, when about 140 grammes, or 4.6 per cent., of a soft, dark-colored extract were obtained. Another lot of the drug, received at a later period, yielded a somewhat smaller amount of extract, namely, 3.3 per cent.

The alcoholic extract was first treated with successive portions of petroleum (b. p. 40–60° C.) until nothing further was dissolved, the liquid filtered, and the petroleum distilled off.

I. EXAMINATION OF THE PETROLEUM EXTRACT.

This extract was an almost black, thick, oily liquid, amounting altogether to 63 grammes. It was brought into a solution of 20 grammes of potassium hydroxide in 200 Cc. of alcohol, and the mixture kept in gentle ebullition for four hours. The alcohol was then distilled off, the alkaline residue diluted with water, and a slight excess of sulphuric acid added, which caused the separation of a considerable amount of light brown,

flocculent precipitate. This was collected on a filter, and washed with water. The filtrate and washings were then distilled, when a slightly acid distillate was obtained, which was treated with barium carbonate, and from the solution of the barium salt a very small amount of a silver salt was prepared.

0.0165 gm. of the salt gave, on ignition, 0.008 gm. Ag.

Ag = 48.5 per cent.

$C_6H_{11}O_2$ Ag requires Ag = 48.6 per cent.

This result would indicate the presence of *caproic acid*, but the amount of substance was much too small to afford any further proof of its identity. The liquid remaining from the distillation of the volatile acid was of a light yellow color, and was shaken out with ether, but the latter yielded only a small amount of an oily, reddish-yellow liquid, which could not be further examined.

The precipitate obtained by the addition of sulphuric acid to the product resulting from the saponification of the petroleum extract, as above described, was dissolved in warm alcohol. On cooling, a considerable portion separated out, but as this apparently consisted of a mixture of substances, the following method for their separation was resorted to: The alcohol was distilled off, and the residue, comprising the whole of the original precipitate, was heated with an aqueous 5 per cent. solution of caustic soda. As the resulting alkaline liquid appeared to contain some uncombined resinous matter which prevented its filtration, it was mixed with clean white sand, evaporated to complete dryness, and extracted in a Soxhlet with petroleum (b. p. 40–60° C.). This removed a considerable amount of a dark-colored substance, the examination of which is described under (b). The residue in the Soxhlet, which would contain all the fatty acids in the form of a soap, was then completely extracted with hot alcohol.

(a) *Identification of the Solid Fatty Acids.*

From the alcoholic solution of the soap just mentioned the alcohol was distilled off, and the residue taken up with water. On acidifying this solution with sulphuric acid a voluminous precipitate was formed, which was dissolved by shaking out the liquid with successive portions of chloroform. The chloroform was then distilled off, and the residue from the latter dissolved in hot alcohol, treated with animal charcoal, and filtered. On standing, a considerable amount of a nearly white, crystalline precipitate separated, which was collected and brought on a porous tile. On recrystallizing from warm petroleum, in which the substance was very sparingly soluble, it was obtained perfectly white. Its melting point was 75° C. Its alcoholic solution was slightly acid to litmus. It was not

acted upon by strong nitric acid, nor colored by sulphuric acid. It was analyzed with the following result :

0.1036 gm. gave 0.2928 gm. CO_2 and 0.1206 gm. H_2O .

C = 77.1; H = 12.9 per cent.

$\text{C}_{20}\text{H}_{40}\text{O}_2$ requires C = 76.9; H = 12.8 per cent.

From the ammonium salt of the acid a silver salt was prepared in the usual manner. This formed a white, amorphous precipitate, which was washed first with water, then with alcohol, and finally dried in a steam oven and analyzed.

0.0988 gm. gave 0.2047 gm. CO_2 , 0.0820 gm. H_2O , and 0.0260 gm. Ag.

C = 56.5; H = 9.2; Ag = 26.3 per cent.

$\text{C}_{20}\text{H}_{39}\text{O}_2$ Ag requires C = 57.3; H = 9.3; Ag = 25.8 per cent.

Another portion of the less pure acid was recrystallized by dissolving it in a very small quantity of warm benzene, and then diluting the solution with petroleum (b. p. 30–40° C.). On allowing the liquid to stand for a few minutes the acid separated in minute glistening crystals, which melted at 74–75° C. This portion of the acid was converted into its silver salt, and the silver determined with the following result :

0.1887 gm. of the salt gave on ignition 0.0488 gm. Ag.

Ag = 25.9 per cent.

$\text{C}_{20}\text{H}_{39}\text{O}_2$ Ag requires Ag = 25.8 per cent.

These results thus indicated that the substance was *arachidic acid*.

For the examination of the mother liquor from which the above mentioned acid was originally obtained, the alcohol was first removed by distillation. The residue was a dark brown semi-solid mass. In order to purify this it was digested on a sand bath with a quantity of 35 per cent. nitric acid for an hour. The cooled mixture was then diluted with water, and the solid acid collected on a filter. It was, however, still too impure to admit of ready crystallization, and the expedient was therefore resorted to of converting it into its amide. This was accomplished by allowing the acid to react on a water bath with an amount of phosphorus trichloride slightly more than that theoretically necessary to convert it into the acid chloride, and then introducing this, drop by drop, into a strong solution of ammonia, kept cool by a freezing mixture. The resulting amide was collected on a filter, and recrystallized from absolute alcohol, from which it separated in a white, crystalline form, having a melting point of 95–96° C. On further crystallization from alcohol its melting point remained unchanged. It was then analyzed with the following result :

(1) 0.1215 gm. gave 0.3382 gm. CO_2 and 0.1442 gm. H_2O .

(2) 0.0757 gm. gave 0.2119 gm. CO_2 and 0.0897 gm. H_2O .

The alcoholic mother liquor from which the above-mentioned amide was obtained still contained a quantity in solution. To this water was added, the precipitate collected on a filter, and recrystallized from absolute alcohol. It then melted at $96-97^\circ \text{C}$., and was analyzed with the following result:

(3) 0.1003 gm. gave 0.2780 gm. CO_2 and 0.1190 gm. H_2O .

Found (1) C = 75.9; H = 13.2 per cent.

(2) C = 76.3; H = 13.2 per cent.

(3) C = 75.6; H = 13.2 per cent.

$\text{C}_{17}\text{H}_{35}\text{CONH}_2$ requires C = 76.3; H = 13.1 per cent.

These results would thus indicate that the acid remaining in the above-mentioned mother liquor from the arachidic acid was *stearic acid*.

(b) *Identification of the Neutral Constituents of the Petroleum Extract.*

As previously mentioned, the final saponified product, mixed with sand, was first extracted in a Soxhlet with petroleum (b. p. $40-60^\circ \text{C}$.) in order to separate any non-saponifiable substances from the associated fatty acids which had been converted into their sodium salts. The petroleum, on standing, deposited a small amount of a crystalline substance. This was recrystallized from absolute alcohol, when it separated in fine white needles, melting at 172°C . On further crystallization from alcohol, and then dissolving in a little warm benzene, with the subsequent addition of petroleum (b. p. $30-40^\circ \text{C}$.), there soon separated a small amount of white needle-shaped crystals, which were at once removed from the mother liquor. The melting point of this substance was 80°C ., and when recrystallized from glacial acetic acid it still melted at $80-81^\circ \text{C}$. After drying for an hour at 115°C . it was analyzed with the following result:

0.0427 gm. gave 0.1273 gm. CO_2 and 0.0550 gm. H_2O .

C = 81.3; H = 14.3 per cent.

$\text{C}_{27}\text{H}_{56}\text{O}$ requires C = 81.8; H = 14.1 per cent.

This substance would therefore appear to be *ceryl alcohol*. (Compare *Journ. Chem. Soc.*, 1890, 57, p. 197, and 1892, 61, p. 916).

The petroleum mother liquor from the above-mentioned crystalline substance which yielded the ceryl alcohol was distilled in order to remove the petroleum, and the residue taken up with alcohol. There then separated from the alcoholic solution a quantity of a white crystalline substance, and on concentrating the mother liquor from this much more of the same substance was obtained. The final syrupy mother liquor was then diluted with much water, which caused the separation of a semi-solid

mass. This was taken up with ether, the ethereal solution washed with water, dried with calcium chloride, and the ether removed by distillation. The residue consisted of a clear, light brown syrup. This was dissolved in warm methylal, and from this solution, after standing for several days, a quantity of crystalline product was obtained. All the crystalline substance thus obtained was then dissolved in absolute alcohol, and to this solution was added the alcoholic mother liquors resulting from the recrystallization of the product which had yielded the above mentioned ceryl alcohol. From this alcoholic solution a fair amount of product was obtained which melted indefinitely between 180° and 194° C., and when digested with a relatively large amount of 90 per cent. alcohol it was observed that a portion remained undissolved. This sparingly soluble portion was collected by the rapid filtration of the hot liquid, and was found to melt at 194° C. The alcoholic filtrate, on cooling, deposited nearly all the substance it contained.

The substance melting at 194° C. was recrystallized from glacial acetic acid. It was then obtained in handsome colorless laminæ, which softened at 205° and melted at 208° C. On again crystallizing from alcohol it formed needles, melting at $207-209^{\circ}$ C. A portion of this substance which had been crystallized from acetic acid, and subsequently allowed to remain in a vacuum desiccator over potash for the night, was placed in a steam oven for a few minutes. It was then observed that it had quickly lost its previous crystalline lustre, and possessed the odor of acetic acid. This was quite in accordance with the property of cholesterol, which is known to form a loose molecular compound ($C_{26}H_{44}O.C_2H_4O_2$) with acetic acid. (Compare Beilstein's *Handbuch der org. Chemie*, ii., p. 1073.)

This highest melting product was finally crystallized from 90 per cent. alcohol in order to remove any combined acetic acid, and was then analyzed with the following result:

0.1059 gm. gave 0.3247 gm. CO_2 and 0.1120 gm. H_2O .

C = 83.6; H = 11.8 per cent.

$C_{26}H_{44}O$ requires C = 83.9; H = 11.8 per cent.

When a small amount of the substance was dissolved in a little acetic anhydride, and a few drops of concentrated sulphuric acid added, a rose-red coloration was gradually developed, which on standing changed to brown.

The alcoholic filtrate from which the sparingly soluble substance, originally melting at 194° C., was obtained, quickly deposited on cooling nearly the entire amount of dissolved substance in the form of white needles melting at 180 to 190° C. This was then crystallized several times from glacial acetic acid, but although in the case of the sparingly

soluble product the melting point shifted from 194 to 208° C. by one crystallization from glacial acetic acid, repeated crystallization of the more freely soluble product from the same solvent failed to raise the melting point above 190–192° C., and this was not altered by a final crystallization from alcohol. It was therefore to be regarded as a distinct and pure substance, and, like that of higher melting point, it formed a loose compound with acetic acid, as will be seen from the following result. A portion which had been recrystallized from glacial acetic acid, from which it separated in glistening laminæ, was first dried on a porous tile, and then allowed to remain for two days in a desiccator over potash :

0.2877 gm. of the substance so treated, when heated to 115° C. until of constant weight, lost 0.0332 gm.

This would correspond to acetic acid = 11.5 per cent.

$C_{26}H_{44}O \cdot C_2H_4O_2$ requires acetic acid = 13.9 per cent.

The amount of combined acid was thus somewhat less than one molecule, a little of the acid having probably become eliminated prior to analysis.

An analysis of the completely dried substance was then made with the following result :

(1) 0.1149 gm. gave 0.3544 gm. CO_2 and 0.1230 gm. H_2O .

A portion of the substance which had been crystallized from glacial acetic acid, and subsequently from alcohol, was also analyzed.

(2) 0.1064 gm. gave 0.3268 gm. CO_2 and 0.1126 gm. H_2O .

Found: (1) C = 84.1; H = 11.9 per cent.

(2) C = 83.8; H = 11.8 per cent.

$C_{26}H_{44}O$ requires C = 83.9; H = 11.8 per cent.

The rotation of the substance in chloroform solution was then determined with the following result :

$c = 1.961$; $l = 1$ dm.; $\alpha_D = +0^\circ 30'$. Hence $[\alpha]_D = +25.5^\circ$.

The color reaction in acetic anhydride solution with concentrated sulphuric acid was the same as that of the higher melting substance.

It is evident that the two crystalline substances above described belong to the class of *cholesterols*, but the observed differences in solubility and in melting point, which exist even after very careful purification, likewise indicate that they represent well differentiated isomers. They do not correspond precisely in their characters to any of the various vegetable substances of this class that have previously been described (compare Beilstein's *Handbuch der org. Chemie*, ii., p. 1075), but it is quite possible that these discrepancies may be due, in some instances, to varying de-

grees of purity. (For a description of some substances of a similar character, see *Ber. d. deutsch. chem. Ges.*, 1891, 24, p. 183, and *Fourn. Chem. Soc.*, 1892, 61, p. 916; 1894, 65, p. 867; 1895, 67, p. 1089.)

Some interesting observations relating to the physiological function of cholesterol in the animal body have recently been recorded by Dr. F. Ransom in a paper entitled: "Saponin und sein Gegengift" (*Deutsch. med. Wochenschrift*, 1901, No. 13, p. 194). After noting the fact that Phisalix has attributed to cholesterol, as also to tyrosin, an immunizing action toward snake poison, and that Fraser has also claimed a similar property for the bile of poisonous snakes, the author remarks as follows:

"The function of cholesterol in the animal economy has hitherto not been quite clear. His observations, however, indicate that it plays an important part in building up the erythrocytes, for its removal or change results in the elimination of the hæmoglobin."

In this connection the same author has studied the hæmolytic action of saponin, chiefly on dogs' blood, and states:

"If, for the purpose of the investigation, the red blood corpuscles be considered as small living masses of protoplasm floating in a dilute serum, it is found that two milligrammes of saponin represent about the minimum lethal dose for the number of these small living bodies contained in 0.7 Cc. of dog's blood. There exists a kind of affinity or relation of solubility between saponin and cholesterol whereby it is possible for the former (saponin) to act as a poison upon tissues containing the latter (cholesterol), but that the latter, under certain circumstances, acts as a protective against the former. So far as the investigation has as yet proceeded, it is found that cholesterol is only active toward actual saponin and the members of the saponin group, and perhaps not toward all of these. Toward other hæmolysines of vegetable origin, as also toward the hæmolytic action of foreign sera, it is, so far as has been tested, quite incapable of affording protection. By the discovery of the relation of cholesterol to the saponin hæmolysis, it has been possible for the first time to isolate, in a state of purity, directly from the tissue attacked by the toxin, the substance which forms the point of attack for the toxin, and at the same time to demonstrate that the same substance may, and actually does, serve as a protective agent."

The observations of Ransom appear to suggest some further inquiries which may be found worthy of investigation, the first and most important of which pertains to the physiological function of the cholesterols occurring in plants. It is interesting, for example, to note in connection with the statement that cholesterol has an immunizing action toward snake poison, that, as mentioned in the introductory part of this paper, of the two closely related species of *Euphorbia* of the Pacific coast—*E. ocellata* and *E. cremocarpus*—the former, although not known to contain cholesterol, is reputed to act as an antidote to snake poison, whereas the latter is used for killing fish. On the other hand it is well known that a large number of plants used as fish poisons contain saponin, whilst the species of *Derris* under examination, which is also used as a fish poison, has been

shown to contain a not inconsiderable amount of isomers of cholesterol. It seems not unlikely that some points of interest, at least in their relation to plant physiology, may be associated with these facts.

II. EXAMINATION OF THE RESINS.

As previously stated, the original alcoholic extract of the drug was first extracted with petroleum of low boiling point. The portion insoluble in the latter was then redissolved in alcohol, and the concentrated solution poured into water in order to precipitate the resin.

The aqueous liquid, filtered from the resin, was concentrated by evaporation. It then afforded no reaction for an alkaloid, thus confirming the previous conclusion that no substance of this class is present in the drug. It contained a considerable amount of *tannin* and *coloring matter*, which were precipitated by lead acetate, but the examination of this precipitate or the filtrate therefrom afforded no substance of special interest. The filtrate contained some *sugar*, which strongly reduced Fehling's solution, and when evaporated and subsequently extracted with alcohol the latter solution, on standing, deposited some crystals of *potassium nitrate*. This inorganic salt appears to be present in relatively large amount in the drug.

The precipitated resin above mentioned formed a flocculent, brown precipitate, which was collected on a filter, washed with water, and dried. It was then brought into a Soxhlet, and completely extracted with chloroform. After distilling off the chloroform the portion of resin that had been dissolved by the latter was found to represent 57.3 per cent. of the whole amount. The undissolved portion, remaining in the Soxhlet, was then extracted with alcohol, the solution concentrated, poured into water, and the precipitated resin collected on a filter, washed with water, and dried. The original resin had thus been resolved into a portion soluble in chloroform, and a portion insoluble in the latter liquid, both of which were separately examined.

1. *Resin Soluble in Chloroform.*

As the alcoholic solution of this resin when added to water formed a soft, sticky mass, it was obtained, after distilling off the chloroform and drying the residue in a water oven, in the form of dark brown scales or fragments, which, when cold, could be reduced to a brown powder.

It contains no nitrogen, and left no ash on ignition. It is quite readily soluble in most of the organic solvents, including glacial acetic acid, but showed no distinct tendency to crystallize from any of these liquids. It was also dissolved to a considerable extent by a 5 per cent. solution of caustic soda, and less readily by a 10 per cent. solution of sodium carbonate, forming yellowish or reddish-yellow solutions. Its alcoholic solution gives a brownish-black color with ferric chloride, and is precipi-

tated by alcoholic solutions of lead acetate and copper acetate, but gives no precipitate with tannic acid. When heated with acetic anhydride and sodium acetate it was not acetylated. A portion of the resin was boiled for an hour with 5 per cent. sulphuric acid, and the acid then removed by digesting with barium carbonate. The filtered liquid had not the slightest reducing action on Fehling's solution, which indicated that this resin is not a glucoside.

Action of Hydrochloric Acid in Alcoholic Solution.

The portion of resin soluble in chloroform appears to be analogous in character to the resinous substance obtained by Greshoff (*loc. cit.*) by a slightly different method from the root of *Derris elliptica*, to which the name of "derrid" has been given, and which is stated to be accompanied by a crystalline substance. A more recent study of "derrid" has been made by Sillevoldt (*loc. cit.*), who, although having obtained it only in an amorphous form, has assigned to it the formula $C_{33}H_{30}O_{10}$, and to the crystalline substance accompanying it, which he regarded as an "anhydroderrid," the formula $C_{33}H_{28}O_9$. As the same crystalline substance was obtained from "derrid" by the action of hydrochloric acid in alcoholic solution, it was assumed to have been produced from it by the action of the acid. The correctness of the formula assigned to "derrid" was, moreover, believed to have been confirmed by the formation of the "anhydroderrid," and by the determination of the methoxyl groups, as it was thus indicated that three such groups are contained in the molecule of both these substances. No molecular weight determination of the crystalline substance appears, however, to have been made, and as there was no direct evidence of the purity of the amorphous "derrid" the deductions made by Sillevoldt from his experiments would hardly seem to be justified.

In view of the above observations it seemed of interest to ascertain whether a similar crystalline substance could be obtained from the portion of resin extracted from *Derris uliginosa*.

Five grammes of the resin were dissolved in 250 Cc. of alcohol, 25 Cc. of hydrochloric acid (sp. grav. 1.115) added, and the mixture heated on a water bath in a flask with inverted condenser for two hours. The liquid became darker in color, and on cooling it was observed that some amorphous resinous matter was suspended in it, while a few small needle-shaped crystals had separated on the sides of the flask. The mixture was filtered at the pump, the separated substance washed with a little alcohol, and brought on a porous tile. On drying, however, it was found to consist chiefly of amorphous matter, and being too impure for further examination it was finally discarded. From the filtered acid liquid the alcohol was distilled off, a quantity of ether added, and subsequently

water, which caused the separation of a considerable amount of hard, dark brown resin, which did not dissolve in the ether and was not further examined. The ethereal layer, however, was separated, washed well with water, dried with calcium chloride, and the ether removed. The residue was chiefly a varnish, but was seen to contain some embedded crystalline substance. The whole was therefore digested with a small quantity of absolute alcohol, which dissolved a dark resinous matter, and after filtration there remained on the filter a greenish-yellow granular substance. This was dissolved in a large amount of hot absolute alcohol, the solution boiled with animal charcoal, filtered, and allowed to cool, when a crop of greenish-yellow needles was obtained. The melting point of this substance was $212-213^{\circ}$ C. It was recrystallized by dissolving in a little chloroform, in which it is very readily soluble, and then adding alcohol. The substance then separated almost immediately in fine sulphur-yellow needles, the melting point of which was the same as before. It was quite insoluble in alkalies, even on warming, and its alcoholic solution gave no coloration with ferric chloride. On analysis it gave the following results:

(1) 0.0956 gm. gave 0.2387 gm. CO_2 and 0.0447 gm. H_2O .

(2) 0.0369 gm. gave 0.0926 gm. CO_2 and 0.0196 gm. H_2O .

(1) C = 68.1; H = 5.2 per cent.

(2) C = 68.4; H = 5.9 per cent.

Although this substance has the same melting point, and appears otherwise to correspond in its physical properties with the "anhydroderrid" described by Sillevoldt (*loc. cit.*), the figures obtained by its analysis are somewhat at variance with his results. It may indeed be considered doubtful whether this substance is actually formed by the action of the hydrochloric acid, for Sillevoldt has found some of the so-called "anhydroderrid" to pre-exist in the resin examined by him, and it is quite possible that the action of the acid simply consists in so changing the character of the amorphous resin as to render the substance associated with it more readily crystallizable. With the hope of obtaining a larger amount of this crystalline substance, and subjecting it to a more complete examination, a decision respecting even its empirical formula may properly be deferred.

The aqueous acid liquid remaining after the extraction of the crystalline substance by ether was concentrated, neutralized and tested for sugar, but with a negative result. This therefore confirms the result obtained by the action of aqueous acid on this resin, and also proves that the crystalline substance is not a hydrolytic product of a glucoside.

Fusion with Potash.

Fifty grammes of caustic potash were dissolved in 50 Cc. of water in a

silver dish, and the solution heated. Ten grammes of the resin were then introduced. It immediately melted, but did not at once dissolve. The heating was continued with a gradual increase of temperature to 200°C . The still undissolved resin then began to react with the caustic potash, frothing ensued, and by maintaining a temperature of 230 to 240°C . for some time, a thick, chocolate-colored homogeneous paste was obtained. This was dissolved in about 300 Cc. of water, the solution acidified with sulphuric acid, and subjected to steam distillation until the distillate no longer had an acid reaction.

The liquid remaining in the distillation flask was cooled, and filtered from a mass of pitch-like substance. The acid filtrate was extracted five times with ether, the ethereal liquid washed once with a little water, dried with calcium chloride, and the ether distilled off, when a very small amount of a brown syrup was obtained. This was purified by digesting with boiling water and treating with animal charcoal, but from the resulting liquid no crystalline product could be obtained. It was observed, however, to give a violet coloration with ferric chloride.

A second portion of 10 grammes of resin was then fused with 75 grammes of caustic potash dissolved in 50 Cc. of water, the operation being conducted as before, but in this case the temperature was allowed to rise to 270 – 280°C ., and was maintained at that point for an hour. After dissolving the mass in water, and acidifying with sulphuric acid, the volatile acids were distilled off, and the residual liquid in the flask treated as before described. In this case also no crystalline product could be obtained.

As noted in the introductory part of this paper, it is recorded that by heating the so-called "derrid" with caustic alkali, Greshoff obtained salicylic and protocatechuic acids. It must remain for the present undecided whether in the case of the resin under examination the violet coloration with ferric chloride is due to the production of salicylic acid or to a phenol. As will be shown later on, the portion of resin which is insoluble in chloroform affords protocatechuic acid on fusion with potash.

The distillates from the acidified solutions of the two potash fusions were combined, made alkaline with sodium carbonate, and the liquid evaporated to a paste. This was acidified with sulphuric acid, and shaken out five times with ether. The ethereal liquid was washed once with a little water, dried with calcium chloride, and the ether removed. The residue was a light brown oil, having an odor reminding of valerianic and acetic acids, and weighed 2.4 grammes. It was converted into a barium salt, and from this a silver salt was prepared, which was washed with a considerable amount of water. The silver salt which remained on the filter was dried and analyzed.

0.0302 gm. of the salt gave on ignition 0.0152 gm. Ag.

Ag = 50.3 per cent.

$C_5H_9O_2$ Ag requires Ag = 51.7 per cent.

The filtrate and washings from this salt were concentrated by evaporation, when two silver salts were collected as they separated during the process of concentration. They were dried and analyzed.

(1) 0.1932 gm. of the salt gave on ignition 0.1194 gm. Ag.

(2) 0.1578 gm. of the salt gave on ignition 0.0992 gm. Ag.

Found (1) Ag = 61.8 per cent.

(2) Ag = 62.9 per cent.

$C_2H_3O_2$ Ag requires Ag = 64.1 per cent.

The volatile acids formed by the fusion of this resin with potash were therefore evidently *valerianic* and *acetic acids*.

Oxidation with Nitric Acid.

Fifteen grammes of the resin with 150 Cc. of 35 per cent. nitric acid were heated to boiling on a sand bath in a flask connected with an inverted condenser for about 8 hours. As oxidation took place the greater part of the resin entered into solution. After several hours boiling the mixture consisted of a clear light yellow fluid with a thin layer of a yellow oil floating on the surface, which did not oxidize further by more prolonged boiling. On cooling, the oily layer became a hard crust. The liquid was filtered, the solid substance spread on a porous tile to dry, and the filtrate set aside for further examination. By repeated crystallization from acetone the solid substance was obtained, in part, in the form of small, white crystals, melting at $75-76^\circ$ C. It contained no nitrogen, and was an acid. The amount of the substance obtained in a pure state was very small, being only 0.3 gramme, but it was evident that much was necessarily lost during the somewhat tedious separation from the uncrySTALLIZABLE matter of which the original solid substance chiefly consisted. The acid was analyzed with the following result:

(1) 0.0920 gm. gave 0.2607 gm. CO_2 and 0.1073 gm. H_2O .

(2) 0.0340 gm. gave 0.0959 gm. CO_2 and 0.0402 gm. H_2O .

Found (1) C = 77.3; H = 13.0 per cent.

(2) C = 77.0; H = 13.1 per cent.

$C_{22}H_{44}O_2$ requires C = 77.6; H = 12.9 per cent.

The substance would thus appear to be *behenic acid*, the melting point of which has been given as $77-78^\circ$ C. With consideration, however, of this being an open-chain acid of 22 carbon atoms, it seems very improbable that it is a product of oxidation, but is more likely to have pre-existed in the resin, possibly in the form of an ester, and to have been separated by the oxidation of substances associated with it. The correctness of this

view is supported by the following subsequent experiment, which also served to indicate the complex composition of the portion of resin soluble in chloroform.

A portion of the resin, for example, was dissolved in ether, and the solution shaken first with several successive portions of a 10 per cent. solution of sodium carbonate and afterwards with a 5 per cent. solution of caustic soda. Both of these liquids were very deeply colored. They were separately acidified with sulphuric acid, which precipitated resinous matter that was taken up by ether, and by the evaporation of the ethereal liquids both of them afforded a dark colored varnish. The original ether solution of the resin from which nothing appreciable was further extracted by the aqueous alkali was relatively light in color. It was washed with water, and the ether removed, when a residue was obtained amounting to nearly 50 per cent. of the original resin. It was dissolved in warm alcohol, and on cooling a mass of indistinctly crystalline matter separated. The entire liquid was then heated for some time with caustic potash, the alcohol distilled off, a little water added, and the strongly alkaline solution shaken out with ether. The latter on evaporation left a residue which, when dissolved in glacial acetic acid, formed a gelatinous mass. The remaining alkaline liquid was then acidified with sulphuric acid, and shaken out with ether. The ethereal liquid left a residue having an odor reminding of fatty acids, and which dissolved in sodium carbonate. These constituent products of the resin were not, however, obtained in sufficient amount for a separate examination.

The nitric acid filtrate from the solid substance which yielded the behenic acid was evaporated on a water bath, with successive additions of water during the operation. The concentrated liquid, on cooling, deposited a quantity of a crystalline substance. This was collected, and dissolved in hot water, in which it was very sparingly soluble, and on cooling a pale yellow crystalline substance was obtained. The mother liquor from the latter afforded on evaporation a crystalline residue consisting entirely of *oxalic acid*. The sparingly soluble substance was recrystallized from much water, and was found to contain nitrogen. It was readily soluble in ammonia and in alkali carbonates, forming solutions of a light yellow color. When dried at 115°C . it melted at $170\text{--}172^{\circ}\text{C}$., and on analysis gave the following results:

- (1) 0.1176 gm. gave 0.1335 gm. CO_2 and 0.0195 gm. H_2O .
- (2) 0.0461 gm. gave 0.0522 gm. CO_2 and 0.0096 gm. H_2O .
- (1) $\text{C} = 30.9$; $\text{H} = 1.8$ per cent.
- (2) $\text{C} = 30.8$; $\text{H} = 2.3$ per cent.
- 0.1089 gm. gave 15.8 Cc. of moist nitrogen at 758 Mm. and 20°C .
- $\text{N} = 16.6$ per cent.

Although from these results an empirical formula has been calculated

for the substance, it has not been possible to identify it. It is the intention, when a larger amount of material can be obtained, to subject it to a more complete study.

2. *Resin Insoluble in Chloroform.*

This was obtained by the precipitation of its concentrated alcoholic solution with water in the form of a chocolate-brown powder. It contains no nitrogen. On ignition it left a small amount of ash. It was fairly soluble in ethyl acetate, acetone, and glacial acetic acid, but showed no tendency to crystallize from these liquids. It was also almost completely dissolved by a 5 per cent. solution of caustic soda, and by a 10 per cent. solution of sodium carbonate, forming dark brown solutions. Its alcoholic solution gives a deep greenish color with ferric chloride, and is precipitated by alcoholic solutions of lead acetate and copper acetate, but gives no precipitate with tannic acid.

Hydrolysis of the Resin.

One gramme of the resin was first heated with 25 Cc. of water for an hour. The liquid frothed considerably, and an aromatic odor was developed, but after filtration it had no reducing action on Fehling's solution. On subsequently heating the residual resin with 5 per cent. sulphuric acid, and removing the acid by barium carbonate, the filtered liquid abundantly reduced Fehling's solution, thus indicating the presence of a *glucoside*.

Five grammes of the resin were then boiled for about 3 hours with 100 Cc. of 5 per cent. sulphuric acid. The filtered liquid, which was of a bright red color, was shaken out several times with ether. On removing the ether a very slight amorphous residue was obtained, the aqueous solution of which gave an olive-green color with ferric chloride. The acid liquid was then digested with barium carbonate until rendered perfectly neutral to test-paper, filtered, and diluted with water to the measure of 100 Cc. It was then of a pale yellow color, and the amount of sugar contained therein was quantitatively determined.

For the reduction of 10 Cc. of Fehling's solution 13.5 Cc. of the above liquid were required. The total amount of sugar in the 100 Cc., calculated as glucose, was therefore 0.37 gramme. The density of the solution was practically 1. Its rotation in a 100 mm. tube was $+0^{\circ} 13'$. From these data the specific rotation of the sugar could be calculated with approximate accuracy, and was found to be $[a]_D = +58.5^{\circ}$. The specific rotation of *d* glucose is accepted as $[a]_D = +52.5^{\circ}$. From a portion of the liquid an osazone was prepared, which, when recrystallized from alcohol, melted at 201° C. The sugar obtained by the hydrolysis was therefore evidently *d* glucose. On the slow evaporation of its solution it formed a pale yellow syrup.

The substance remaining from the hydrolysis of the 5 grammes of resin was but little altered in appearance, and when washed and dried weighed 4.3 grammes. It was then mixed with clean sand and extracted in a Soxhlet with chloroform. A small amount of substance was thus extracted, which was evidently a product of hydrolysis, as the original resin was quite insoluble in chloroform. On allowing this liquid to evaporate, the residue was observed to contain a few crystals. It was redissolved in a little alcohol, and by this treatment a very small amount of a substance was obtained which crystallized in colorless, micaceous scales, and melted at about 230° C. It gave no coloration with ferric chloride, and was too small in amount for further examination.

The resin remaining in the Soxhlet was then extracted with alcohol. To the very dark colored liquid 1 Cc. of hydrochloric acid, sp. gr. 1.16, was added, and the mixture boiled for 2 hours. The concentrated liquid was then poured into water to precipitate the resin, and filtered. After neutralization it did not reduce Fehling's solution, thus proving that no further hydrolysis had taken place after the action of the aqueous acid. From the comparatively small amount of sugar first obtained it may also be concluded that the glucosidal constituent of the resin which affords it represents but a small proportion of the latter.

Fusion with Potash.

Fifty grammes of caustic potash were dissolved in 50 Cc. of water in a silver dish, the solution heated, and 10 grammes of the resin introduced. On continuing the heat the resin dissolved, and the temperature was allowed to gradually rise to 220° C. It was kept at this point until the melt became a thick paste. This was then dissolved in about 300 Cc. of warm water, the liquid acidified with sulphuric acid and steam distilled.

The distillate, which was practically odorless, had a strongly acid reaction, and sodium carbonate was added until rendered faintly alkaline. It was then evaporated nearly to dryness, acidified with sulphuric acid, and extracted five times with ether. The ethereal solution was washed once with a little water, dried with calcium chloride, and the ether removed. The residue, which weighed 0.8 gramme, was a light brown, pungent-smelling liquid, suggestive of acetic acid. It was converted into its barium salt, and from the latter a silver salt was precipitated in two fractions. These silver salts were nearly white, dissolved readily in hot water, and on cooling separated in a crystalline form. They were analyzed with the following results:

Fraction I. 0.0873 gm. of the salt gave on ignition 0.0535 gm. Ag.

Fraction I. 0.1779 gm. of the salt gave on ignition 0.1097 gm. Ag.

Fraction II. 0.1680 gm. of the salt gave on ignition 0.1068 gm. Ag.

Found—Fraction I. 61.3; 61.6. Fraction II. 63.6 per cent. Ag.

$C_2H_3O_2$ Ag requires 64.1 per cent. Ag.

The volatile acid formed by the fusion was therefore essentially *acetic acid*.

The liquid remaining from the distillation of the volatile acid was extracted five times with ether, the ethereal solution washed once with a little water, dried with calcium chloride, and the ether removed by distillation. The residue was a dark brown syrup. This was poured into about 200 Cc. of boiling water, the mixture digested for half an hour and filtered. The brownish colored filtrate was boiled with animal charcoal, again filtered, and evaporated to a very small bulk. This on cooling became a crystalline paste, which was spread on a porous tile. The substance was then recrystallized from water, from which it separated in nearly colorless needles, having a melting point of 196° C. The aqueous solution of these crystals gave, on the addition of a drop of ferric chloride, a greenish-blue coloration, which was changed to bluish-violet on the subsequent introduction of one drop of sodium carbonate solution, and to blood-red when more of the same reagent was added.

0.1415 gm. of the air-dried substance when heated at 115° C. until of constant weight lost 0.0150 gm., or 10.6 per cent. H_2O .

$C_7H_6O_4 \cdot H_2O$ requires 10.5 per cent. H_2O .

0.1265 gm. of the substance dried at 115° C. gave on analysis 0.2526 gm. CO_2 and 0.0458 gm. H_2O . C = 54.5; H = 4.0 per cent.

$C_7H_6O_4$ requires C = 54.5; H = 3.9 per cent.

The crystalline substance produced by this fusion was therefore identified as *protocatechuic acid*.

Oxidation with Permanganate.

Five grammes of the resin were dissolved in the cold in a 10 per cent. solution of caustic soda, and a saturated cold solution of potassium permanganate added from time to time as decolorization ensued. This was continued until the red color of the liquid remained permanent for a day. On working up the liquid, however, in the usual way, the only crystalline product that could be isolated was *oxalic acid*.

PHYSIOLOGICAL ACTION OF DERRIS AND ITS RESINS.

The experiments here described were conducted only upon fish.

Ten grammes of the ground bark, free from the woody portion of the stem, were digested with cold water for 24 hours, the dark reddish colored liquid strained, and diluted with water to the measure of 100 Cc.

1. Five Cc. of the above liquid were mixed with 1000 Cc. of water, and a small gold-fish introduced. It seemed at first to be stupefied, but after 15 minutes became more active, and in half an hour appeared to have quite recovered. After remaining for about 2 hours in the liquid, and in the meantime not being further affected, it was then transferred

to fresh water, when it continued to remain in a perfectly normal condition for days.

2. The experiment was repeated with a somewhat larger gold-fish, but in this instance 20 Cc. of the above liquid were mixed with 1000 Cc. of water. The fish soon appeared to be stupefied, and after three-quarters of an hour became motionless and sank to the bottom of the vessel. It revived temporarily, but after 3 hours floated on its side on the surface of the water, and was soon dead.

3. In view of the recent statement of Kyle (*loc. cit.*) respecting the toxic action of tannic acid on fish, it seemed of interest to make a comparative experiment with this substance, especially as the bark of Derris is particularly rich in tannin. As the amount of tannin in the bark employed for the preceding experiments was known, a solution of pure tannic acid (Kahlbaum's) of the same calculated strength as the above-mentioned liquid was prepared, that is, 0.93 gramme of tannic acid in 100 Cc. of water. 20 Cc. of this solution were mixed with 1000 Cc. of water and a gold-fish introduced. The water almost immediately became milky throughout, to such an extent finally as to almost completely obscure the fish. This interesting phenomenon was evidently due to something ejected by the fish, and which possibly reacted with the tannic acid, although the liquid in experiment 2, which contained by calculation the same amount of tannic matter, remained perfectly clear. The fish was at first active, but after half an hour became quite motionless, and remained at the bottom of the vessel. It subsequently revived, and after remaining in the liquid for four hours it was brought into fresh water, when it continued to remain in a perfectly normal condition for days. This considerable amount of tannic acid therefore did not prove destructive to the fish, and its action was in general very different from that produced by the corresponding infusion of Derris.

The action of the two resins obtained from the Derris was now determined. For this purpose solutions were prepared of the same strength as that employed by Sillevoldt (*loc. cit.*) in his experiments with the so-called "derrid" from *Derris elliptica*.

4. *Resin soluble in chloroform.* 0.01 gramme of this resin was dissolved in 5 Cc. of absolute alcohol, and the solution diluted with water to the measure of 100 Cc. 10 Cc. of this dilute and slightly opalescent liquid were mixed with 1000 Cc. of water, and a gold-fish introduced. The fish did not appear to be much affected at first, but in the course of 3 hours it was completely stupefied, and remained motionless when touched. It could not be further observed until the following morning, when it was lying on its side on the surface of the water practically lifeless, and did not revive when brought into fresh water. It will be observed that the amount of substance which produced this effect corresponded to 1 part of the resin in one million parts of water.

5. *Resin insoluble in chloroform.* This resin was tested in precisely the same manner and at the same time as the preceding. The fish, however, was entirely unaffected, even after remaining in the liquid for 24 hours. It was then brought into fresh water, and continued to remain in a perfectly normal condition for days.

Although this last result showed that the amount of alcohol contained in the liquid was not sufficient to produce any action on the fish, yet, for the purpose of control, another gold-fish was allowed to remain during the time of these experiments in a liquid containing the same amount of alcohol as was present in the water containing the resins. It was not in the least affected at any time, and after remaining in the liquid for 24 hours it was brought into fresh water.

The results of the preceding experiments, especially those described under 2 and 4, not only confirm the reputed poisonous action of this species of *Derris* on fish, but they would also seem to have demonstrated that the stupefying or toxic effect is produced by some constituent of that portion of the resin which is soluble in chloroform, and which is naturally contained in small amount in a cold aqueous infusion of the drug. The exceedingly small amount of the resin that is required to produce a fatal effect, together with the fact that the original resin had been precipitated with water, and that the active portion had been obtained from this precipitated crude resin by extraction with chloroform, renders it impossible to assume that the tannic acid contained in the drug can play any important part in its action as a fish poison, as the previously mentioned observations of Kyle might otherwise lead one to infer. Moreover, the "derrid" obtained by Sillevoldt from *Derris elliptica* appears to have been even more powerful in its action than the corresponding resin from *Derris uliginosa*, and as he has stated that its alcoholic solution gave no coloration with ferric chloride, it could not have contained any tannin.

Summary and Conclusions.

The somewhat extended details of the experimental work described in the preceding pages suggests the following summary of the essential results:

1. It has been stated that the bark of *Derris uliginosa* contains an alkaloid, but no evidence could be obtained of the presence of such a substance in the stems of the plant now examined.

2. The drug contains a considerable amount of *tannin* and *red coloring matter*. A quantitative determination of the tannin has shown this to exist to the extent of 9.3 per cent. in the bark, corresponding to 6.6 per cent. in the entire stem. It is the variety which gives a greenish coloration with ferric chloride.

Besides *gum* and *sugar*, the stems contain an appreciable amount of

inorganic salts, notably *potassium nitrate*. The more important constituents, however, are those enumerated below, in connection with which some of the products afforded by them are also noted.

3. *Constituents of the Petroleum Extract*. The original alcoholic extract of the drug, when extracted by petroleum (b. p. 40–60° C.), afforded a considerable amount of a very dark colored, oily liquid. This was hydrolyzed by heating with alcoholic potash. The portion of the hydrolyzed product which had entered into combination with the alkali yielded a very small amount of a volatile acid, the silver salt of which gave figures agreeing with *caproic acid*, $C_6H_{12}O_2$. A crystalline acid was also obtained, having a melting point of 74–75° C., and agreeing in composition with *arachidic acid*, $C_{20}H_{40}O_2$. The mother liquors from the latter contained an acid which was identified by the analysis of its crystalline amide, m. p. 95–96° C., as *stearic acid*, $C_{18}H_{36}O_2$.

The portion of the hydrolyzed product which had not entered into combination with the alkali was found to contain a small amount of *ceryl alcohol*, $C_{27}H_{56}O$, m. p. 80–81° C., and a considerable quantity of two isomers of *cholesterol*, $C_{26}H_{44}O$. One of these, which occurred in relatively small amount and was sparingly soluble in 90 per cent. alcohol, had a melting point of 207–209° C., while the one constituting the larger portion was more freely soluble in alcohol, and melted at 190–192° C. The optical rotation of the latter, in chloroform solution, was $[\alpha]_D = +25.5^\circ$. When dissolved in a little acetic anhydride, and a few drops of concentrated sulphuric acid added, both of them gradually developed a rose-red coloration, changing to brown.

The original resin, which had been extracted as above mentioned by petroleum, was redissolved in alcohol, precipitated by water, and dried. It was then extracted with chloroform, and thus resolved into portions soluble and insoluble therein.

4. *Resin soluble in chloroform*. This was an amorphous substance, which contained no nitrogen, and was not a glucoside. By the action of hydrochloric acid in alcoholic solution it afforded a small amount of a substance which crystallized in fine yellow needles, was very sparingly soluble in cold alcohol, and melted at 212–213° C. This would appear to be identical with the so-called “anhydroderrid” of Sillevoldt, but on analysis it gave somewhat different figures from those recorded by him. It is the intention to further examine this substance.

On fusion with potash this resin afforded *acetic* and *valerianic acids*, and a very small amount of a substance giving a violet coloration with ferric chloride. By the oxidation of the resin with nitric acid a small amount of a crystalline acid was obtained, melting at 75–76° C., and agreeing in composition with *behenic acid*, $C_{22}H_{44}O_2$. It is probable, however, that the latter pre-existed as such or in some form of combina-

tion in the resin. Other products of the oxidation were *oxalic acid*, and a pale yellow, crystalline substance, which, when dried at 115°C ., melted at $170\text{--}172^{\circ}\text{C}$. This was a nitro product, and it was analyzed, but a decision regarding its constitution will be deferred until it can be more completely examined.

5. *Resin insoluble in chloroform*. This was obtained by the precipitation of its alcoholic solution with water in the form of an amorphous, chocolate-brown powder, which contained no nitrogen. Its alcoholic solution was observed to froth considerably when shaken with water. When heated with 5 per cent. sulphuric acid the filtered liquid was found to contain glucose, which was identified by means of its osazone. From the residual resin chloroform extracted a small amount of a substance, which, after purification by means of alcohol, crystallized in colorless, micaceous scales, melting at about 230°C . The amount of this substance was not sufficient for further examination. It was evident, however, from the small amount of sugar obtained by the hydrolysis that only a portion of this resin consisted of a glucoside.

On fusion with potash this resin afforded *acetic* and *protocatechuic acids*. When oxidized in a cold alkaline solution with potassium permanganate, the only crystalline product that could be isolated was *oxalic acid*.

6. The poisonous action of *Derris* on fish may be observed when a cold aqueous infusion of the bark is mixed with a relatively large portion of water. The toxic effect, however, is evidently due to some constituent of that portion of the resin which is soluble in chloroform, and not to the tannin which the drug contains. This was demonstrated by the stupefying and finally fatal effect produced on a gold-fish when brought into a liquid containing so little of the active substance as was represented by one part of the resin in one million parts of water. The portion of resin insoluble in chloroform, when tested under precisely the same conditions, was quite devoid of activity.

It is hoped that it may be possible at some future time, and with a larger quantity of material, to pursue the investigation of these resins from *Derris uliginosa*, especially with reference to the constitution of some of the crystalline substances that have been obtained from them, and also to compare these with the constituents of the closely allied plant—*Derris elliptica*.

Finally, I desire to express my indebtedness to Mr. Frederic H. Lees and to Mr. Frank Shedden, of the laboratory staff, for the very valuable assistance they have given me in conducting this investigation.

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